

Solving Research Problems: Investigating Transcriptional Networks in Human Glioma

Molecular Analysis Tools Knowledge Center

Molecular analysis tools provide powerful analytic capabilities for genomic data interpretation. Four key tools are supported by the Molecular Analysis Tools Knowledge Center: geWorkbench, which provides an innovative, open-source software platform for genomic data integration while bringing together analysis and visualization tools for gene expression, sequences, pathways and other biomedical data; GenePattern, which provides bioinformatics tools for gene expression, proteomic and SNP analysis; caArray, a system that supports the management and exchange of array data and annotations and caIntegrator, a novel translational informatics platform that allows researchers and bioinformaticians to access and analyze clinical and experimental data across multiple clinical trials and studies.

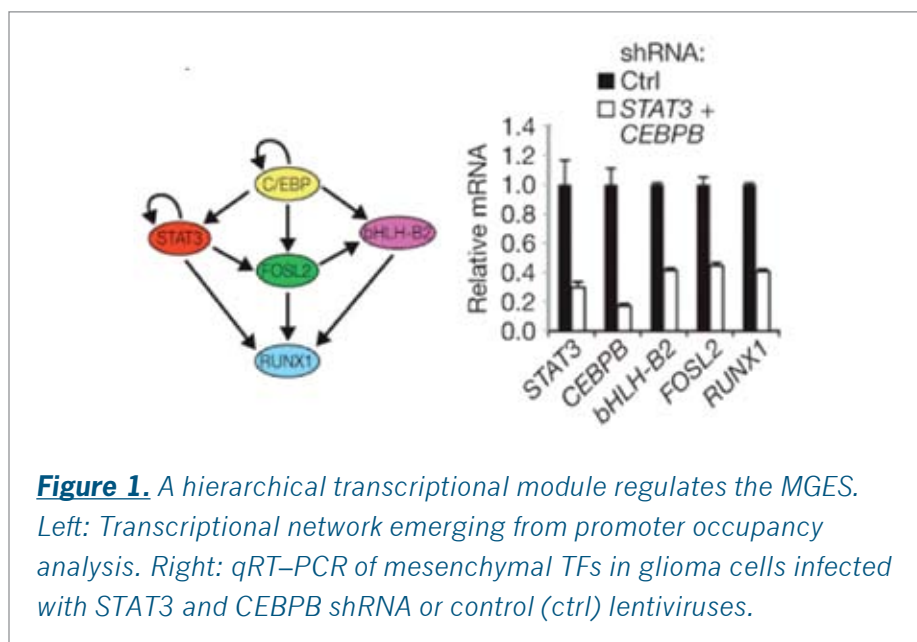
Problem Statement: Identifying Key Transcriptional Regulators

Which transcription factors control the ‘mesenchymal’ phenotypic transformation in aggressive, high-grade glioma in humans? Can the associated gene regulatory program be discerned from large-scale human cancer gene expression data sets?

Methods: ARACNe and geWorkbench

ARACNe (Algorithm for the Reconstruction of Accurate Cellular Networks) (Basso 2005, Margolin 2006a, 2006b) is an algorithm that uses gene expression profiles to calculate the mutual information between pairs of genes, for example between transcription factors (TFs) and their targets. By proper selection of samples, a tissue/phenotype-specific set of pairwise regulatory interactions can be obtained – an “interactome.” Such an interactome can form the basis for more complex analysis of cellular regulatory networks.

Master Regulator analysis (Lefebvre 2010) is an



algorithm used to identify transcription factors whose targets (e.g., as represented in an ARACNe-generated interactome) are enriched for a particular gene signature. The enrichment is evaluated using a statistical test such as Fisher's exact test.

ARACNe is available both as a stand-alone program and as a component in geWorkbench, an open-source platform for integrative genomics (Floratos 2010). Master Regulator Analysis is also available as a component in geWorkbench.

Results:

The mesenchymal phenotype is the hallmark of tumor aggressiveness in human malignant glioma, associated with an uncontrolled ability to invade and stimulate angiogenesis. Gene expression studies (Phillips 2006) have established that overexpression of a 'mesenchymal' gene expression signature (MGES) co-segregates with the poor prognosis. Using reverse-engineering and an

unbiased interrogation of the resulting glioma-specific regulatory network, Carro et al. (2010) identified the transcriptional module that activates expression of MGES. ARACNe was applied in the first step of the analysis, to generate a context-specific interactome for high-grade glioma. Master Regulator Analysis was then used to prioritize all transcription factors based on the enrichment score of their ARACNe-predicted target sets for MGES. This approach allowed the identification of a small regulatory

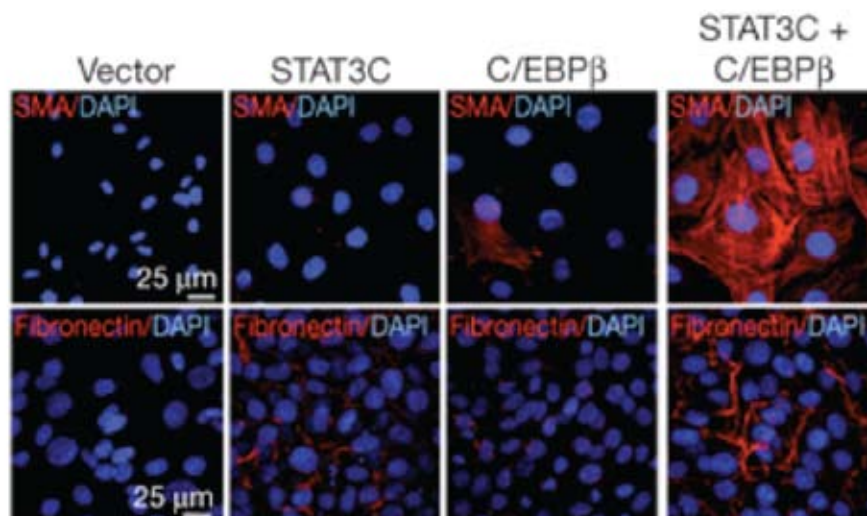


Figure 2. Immunofluorescence analysis in C17.2 cells shows that joint ectopic expression of STAT3 and C/EBPβ leads to gain of expression of the mesenchymal marker proteins smooth muscle alpha actin (SMA) and fibronectin (while ectopic expression of either STAT3 or C/EBPβ alone has a much smaller effect).

module of 6 TFs, with C/EBPβ and STAT3 on top of the hierarchy, as master regulators of the mesenchymal signature (Figure 1). Further experimental validation showed that activation of C/EBPβ and STAT3 was necessary and sufficient to support the transition to the aberrant phenotypic state (Figures 2, 3). These results illustrate the power of ARACNe and how it can be integrated in more complex analyses for addressing a specific biological question. The same type of analysis is now being repeated for the study of other phenotypic states.

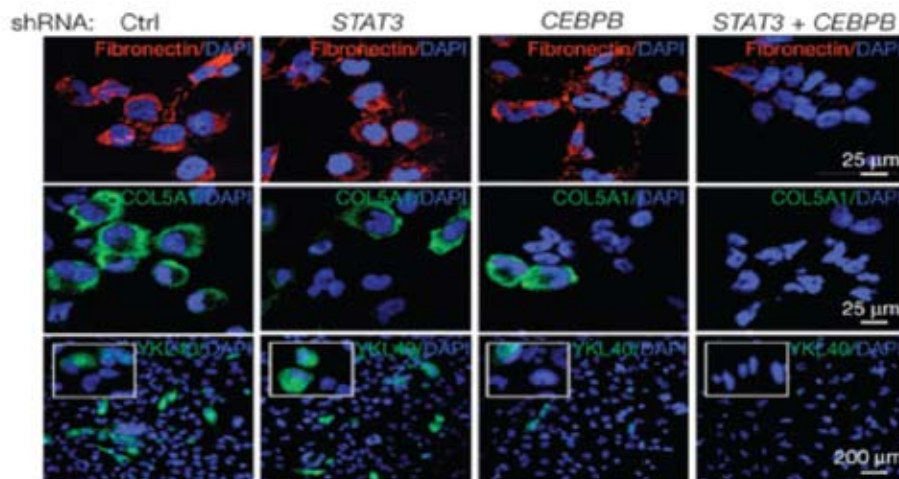


Figure 3. Immunofluorescence for fibronectin, collagen-5 α 1 (COL5A1) and YKL40 in BTSC-3408 infected with lentiviruses expressing STAT3, CEBPB or STAT3 plus CEBPB shRNA, showing how joint shRNA-induced silencing of STAT3 and C/EBPB in human GBM cell lines presenting the mesenchymal phenotype can lead to elimination of mesenchymal markers.

Key Contributors:

Computational analysis: Andrea Califano, Wei Keat Lim, Mariano Alvarez
ARACNe, Master Regulator Analysis: Califano group, Columbia University.

For More Information:

Molecular Analysis Tools Knowledge Center:

https://cabig-kc.nci.nih.gov/Molecular/KC/index.php/Main_Page

References:

- Basso K, Margolin AA, Stolovitzky G, Klein U, Dalla-Favera R, Califano A (2005) Reverse engineering of regulatory networks in human B cells. *Nat Genet.* 37(4):382-390
- Carro MS, Lim WK, Alvarez MJ, Bollo RJ, Zhao X, Snyder EY, Sulman EP, Anne SL, Doetsch F, Colman H, Lasorella A, Aldape K, Califano A, Iavarone A (2010) The transcriptional network for mesenchymal transformation of brain tumors. *Nature* 463(7279):318-25.
- Floratos A, Smith K, Ji Z, Watkinson J, Califano A (2010) geWorkbench: an open source platform for integrative genomics. *Bioinformatics* 26(14):1779-80.

Lefebvre C, Rajbhandari P, Alvarez MJ, Bandaru P, Lim WK, Sato M, Wang K, Sumazin P, Kustagi M, Bisikirska BC, Basso K, Beltrao P, Krogan N, Gautier J, Dalla-Favera R, Califano A (2010) A human B-cell interactome identifies MYB and FOXM1 as master regulators of proliferation in germinal centers. *Mol Syst Biol.* 6:377. PMID: 20531406

Margolin AA, Nemenman I, Basso K, Wiggins C, Stolovitzky G, Dalla Favera R and Califano A (2006a) ARACNE: An Algorithm for the Reconstruction of Gene Regulatory Networks in a Mammalian Cellular Context, *BMC Bioinformatics* 7(Suppl.1):S7

Margolin A, Wang K, Lim WK, Kustagi M, Nemenman I, and Califano A (2006b) Reverse Engineering Cellular Networks. *Nature Protocols* 1(2):663-672

Phillips HS, Kharbanda S, Chen R, Forrest WF, Soriano RH, Wu TD, Misra A, Nigro JM, Colman H, Soroceanu L, Williams PM, Modrusan Z, Feuerstein BG, Aldape K (2006) Molecular subclasses of high-grade glioma predict prognosis, delineate a pattern of disease progression, and resemble stages in neurogenesis. *Cancer Cell* 9(3):157-73.

